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GENERAL DIRECTOR

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STORROW HOUSE
(CONVALESCENTS)

October 2, 1956

Dear Dr. Lederberg:

One of the cultures which I sent you, the Proteus 52 regained from the L form, needs some comment. It has a high resistance to penicillin and the culture is pleomorphic on penicillin plates, but most of the colonies are not of the L-type. In the same way, it is difficult to decide the nature of the growth in broth. Such strains may be interesting in some respects but are not appropriate to study the properties of the L forms and it was a mistake to send it to you. Usually the resistance to penicillin of a Proteus recovered from the L form is not increased.

I would like to add a few remarks to the possible connection of protoplasts, large bodies and L forms. My impression is that if we regard the protoplasts as the living organisms inside of a fairly rigid cell wall, the L forms are probably the growth of this organism without building up the cell wall. However, the large body is not the protoplast of a single bacterium but it is produced by a limited multiplication of a single protoplast. This was most clearly visible with the Bacteroides strain 132 to which I will refer in an other connection also. The large bodies developed in the broth cultures of this strain without the addition of penicillin or any other growth-inhibiting substances. The sequence was thus and almost all bacteria went through the same sequence at the same time:-

Division stopped after 6 - 8 hours.
When transfers were made to an agar plate during the development of large bodies, within a few hours all these various forms were retransformed to the usual bacilli. The most interesting was the retransformation of :-

They fall without changing the outline of the structure into bacilli indicating that the bacilli were preformed in them:-
I published photographs of this process. It is apparent in this case that the development of the large bodies was not a physical process like inhibition with water, but a growth process involving division of the living units. The staining of the chromatinic structures both in strain 132 and in other bacteria indicated the same thing.

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In strain 132 it was very interesting to observe this process. At about 4 hours most bacilli grew to short filaments about 2-4 bacilli long. The formations of the large body started by an incomplete division:-

The next step was juxtaposition and fusion of the segments- producing forms surprisingly similar to those which you desc ribed in B. coli. Usually this fusion was complete but in some cases a dividing line remained between the structures developing from the two bacilli:-

Dr. Smith who worked with me wrote a short paper about these forms. A fusion preceding the formations of large bodies is visible also sometimes in B. coli and Salmonella in the following way: First a quadrangular side growth develops in the filaments and later this fuses into a large body:-

At present, when sexual intermixture of the characteristics and conjugal paring also have been observed, these few observations concerning the origin of large bodies seem significant. Maybe they will lead to a better understanding of what the large bodies really are.

The growth of the L forms from the large bodies does not appear to me a budding similar to that of yeast. A large body, as is apparent from the way it is formed, does not consist of one but of many living units and these living units grow out of it. In photograph these chromatinic bodies are stained. They look about the same in the large bodies as in the bacteria only that they are arranged in a sphere. When the large body grows further (photograph I) the chromatinic bodies multiply. The old photograph (II,III) shows the way of growth and the size of the growing elements of the cultures. These are smaller than the bacteria but not too much smaller. The smallest are about 0.3 to 0.5 μ. In most cultures they are larger and may swell to large forms. Their arrangement in the culture indicates that they multiply like bacteria. In electron micrographs from Proteus L, there are many granules between 0.15 to 0.3 μ and some less than 0.1 μ. Thus far the multiplication of single granules of this size has not been observed. Growth can be obtained in our media only from large inoc ula. This may explain why the small granules do not grow.

The growth cycle in large colonies of certain cultures seems to be different and to consist of growth of the small granules into large bodies and the subsequent breaking-up of these again into granules without multiplication of the small granules in such form.

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As far as I can see, there is nothing in the origin and multiplication of L forms which contradicts the supposition that they correspond to protoplasts. They may be protoplasts slightly modified or in a special condition. They are poorly adapted to ouf media and most cells die and disintegrate.

Would you mind if I made a few experiments with the two colon bacillus strains in which mating is often observed? If not, could you send me these two strains?

With kind regards,

Sincerely yours,

Louis Dienes

Louis Dienes, M.D.

I received your letter of September 27 after this letter was typed. I would like very much to read the promised enclosure but it was not in the letter. It is wonderful that you succeeded to get L forms from K-12. I had some time ago the idea that the L cultures may be analogous to the haploid yeast colonies of Winge. It will be very interesting to see whether they have any genetic function.

It may be of some use if I also make a few experiments with your colon bacillus strains. I would be very much interested to know ~~whether~~ what kind of L cultures you get. I leave it entirely up to you whether it fits into your plans or not.

I am quite excited to learn how your experiments are developing.